# Novel variants at *KCTD10*, *MVK*, and *MMAB* genes interact with dietary carbohydrates to modulate HDL-cholesterol concentrations in the Genetics of Lipid Lowering Drugs and Diet Network Study[1–4]

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## ABSTRACT

**Background:** Several genome-wide association studies have identified novel loci (*KCTD10*, *MVK*, and *MMAB*) that are associated with HDL-cholesterol concentrations. Of the environmental factors that determine HDL cholesterol, high-carbohydrate diets have been shown to be associated with low concentrations.

**Objective:** The objective was to evaluate the associations of 8 single nucleotide polymorphisms (SNPs) located within the *KCTD10*, *MVK*, and *MMAB* loci with lipids and their potential interactions with dietary carbohydrates.

**Design:** *KCTD10*, *MVK*, and *MMAB* SNPs were genotyped in 920 subjects (441 men and 479 women) who participated in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study. Biochemical measurements were made by using standard procedures. Dietary intakes were estimated by using a validated questionnaire.

**Results:** For the SNPs *KCTD10*_i5642G→C and *MVK*_S52NG→A, homozygotes for the major alleles (*G*) had lower HDL-cholesterol concentrations than did carriers of the minor alleles ($P = 0.005$ and $P = 0.019$, respectively). For the SNP 12inter_108466061A→G, homozygotes for the minor allele (*G*) had higher total cholesterol and LDL-cholesterol concentrations than did *AG* subjects ($P = 0.030$ and $P = 0.034$, respectively). Conversely, homozygotes for the major allele (*G*) at *MMAB*_3U3527G→C had higher LDL-cholesterol concentrations than did carriers of the minor allele ($P = 0.034$). Significant gene-diet interactions for HDL cholesterol were found ($P < 0.001$–$0.038$), in which *GG* subjects at SNPs *KCTD10*_i5642G→C and *MMAB*_3U3527G→C and *C* allele carriers at SNP *KCTD10*_V206VT→C had lower concentrations only if they consumed diets with a high carbohydrate content ($P < 0.001$–$0.011$).

**Conclusion:** These findings suggest that the *KCTD10* (V206VT→C and i5642G→C) and *MMAB*_3U3527G→C variants may contribute to the variation in HDL-cholesterol concentrations, particularly in subjects with high carbohydrate intakes. *Am J Clin Nutr* 2009;90:686–94.

## INTRODUCTION

Low concentrations of HDL cholesterol have been shown to be associated with an increased risk of coronary heart disease (CHD) (1). Of the environmental factors that determine plasma HDL-cholesterol concentrations, high-carbohydrate diets have been shown to be associated with lower concentrations (2–4). However, not all individuals exposed to similar environmental factors have low HDL-cholesterol concentrations, reinforcing the possibility that variation in genetic susceptibility may influence HDL-cholesterol concentrations. In this regard, family and twin studies have shown that 50% of the variation in HDL-cholesterol concentrations is genetically determined (5, 6). Therefore, the variation in HDL cholesterol depends on the joint action of genetic and environmental factors and their interaction.

Recent genome-wide association analyses (GWAS) have discovered novel loci at chromosome 12q24, which includes the genes *MVK* (murine mevalonate kinase), *MMAB* [methylmalonic aciduria (cobalamin deficiency) cbIB type], and *KCTD10* (potassium channel tetramerization domain-containing 10), all of which influence HDL-cholesterol concentrations (7, 8). The importance of this region in influencing lipid concentrations has also been reported by other linkage studies (9–11). In particular, 2 neighboring genes—*MVK* and *MMAB*—participate in metabolic pathways associated with HDL metabolism (12). Mevalonate kinase, encoded by *MVK*, catalyzes an early step in cholesterol biosynthesis. In humans, homozygosity for milder

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*MVK* mutations produces hyperimmunoglobulinemia D syndrome, which is characterized by fever and increased concentrations of immunoglobulins D and A. In agreement with GWAS findings (7, 8), patients with hyperimmunoglobulinemia D syndrome have low HDL-cholesterol concentrations. In humans, deficiency of cob(I) alamin adenosyltransferase, an enzyme encoded by *MMAB*, results in methylmalonic aciduria (13). Although the role of *MMAB* in cholesterol metabolism remains unclear, one study showed a negative correlation between urinary methylmalonic acid and red blood cell membrane cholesterol concentrations in patients with schizophrenia (14). In close proximity to the *MVK* and *MMAB* genes, the *KCTD10* gene has been shown to have membership in a gene network perturbed by loci contributing to the susceptibility of obesity, diabetes, and atherosclerosis (15). Because there remains some discrepancy about which genes direct the associations with HDL-cholesterol concentrations, it is important to assess the association of different markers, each of which represents distinct regions of linkage disequilibrium (LD).

Only one of the previous studies that examined this chromosomal region with chosen variants has investigated the effects of *MVK*_S52NG→A polymorphism on HDL-cholesterol concentrations (16), but the contribution of dietary components was not assessed. Furthermore, diet was not considered in the GWAs described above (7, 8). Therefore, the aims of the present study were first to assess the association of several polymorphisms at the *KCTD10, MVK,* and *MMAB* genes with lipids, particularly with HDL cholesterol. Second, we investigated whether these genetic variants interact with dietary carbohydrates to modulate HDL-cholesterol concentrations.

## SUBJECTS AND METHODS

### Subjects

The study population ($n$ = 920) consisted of 441 men and 479 women aged 49 ± 16 y who participated in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study. Participants were recruited from 3-generational pedigrees from 2 National Heart, Lung, and Blood Institute Family Heart Study field centers (Minneapolis, MN, and Salt Lake City, UT) (17). The study population was homogeneous with regard to ethnic background, ie, all individuals were of European origin. The detailed design and methodology of the study were described previously (18). The protocol was approved by the Institutional Review Boards at the University of Alabama, the University of Minnesota, the University of Utah, and Tufts University. Written informed consent was obtained from each participant.

### Data collection

For GOLDN participants, clinical examinations at the baseline visit included anthropometric and blood pressure (BP) measurements. Weight was measured with a beam balance and height with a fixed stadiometer. Body mass index (BMI) was calculated as weight (in kg) divided by the square of height (in m). BP was measured twice with an oscillometric device (Dinamap Pro Series 100; GE Medical Systems Information Technologies and Critikon Company, LLC, Tampa, FL) while the subjects were seated after having rested for 5 min. Reported systolic and diastolic BP values were the mean of 2 measurements. Questionnaires were administered to assess demographic and lifestyle information, medical history, and medication use. Physical activity was considered starting from an activity 2 times/wk during a minimum of 2 h.

Habitual dietary food intake was assessed by using the Diet History Questionnaire developed by the National Cancer Institute (19). It consists of 124 food items and included portion size and dietary supplement questions. Two studies have confirmed its validity (20, 21).

### Laboratory methods

Blood samples were drawn after the subjects had fasted overnight. Fasting glucose was measured by using a hexokinase-mediated reaction, and total cholesterol was measured by using a cholesterol esterase cholesterol oxidase reaction on a Hitachi 911 autoanalyzer (Roche Diagnostics, Indianapolis, IN). The same reaction was used to measure HDL cholesterol after precipitation of non-HDL cholesterol with magnesium/dextran. LDL cholesterol was measured by using a homogeneous direct method (LDL Direct Liquid Select Cholesterol Reagent; Equal Diagnostics, Exton, PA). Triglycerides were measured with a glycerol-blanked enzymatic method on the Roche COBAS FARA centrifugal analyzer (Roche Diagnostics).

### Genetic analyses

DNA was extracted from blood samples and purified by using commercial Puregene reagents (Gentra Systems, Minneapolis, MN) following the manufacturer's instructions. Eight single nucleotide polymorphisms (SNPs) within the *KCTD10, MVK*, and *MMAB* loci (*KCTD10*_V206VT→C, rs2302706; *KCTD10*_i5642G→C, rs10850219; 12inter_108466061A→G, rs731178; *MMAB*_3U3527G→C, rs2241201; *MVK*_m751T→C, rs12314392; *MVK*_i851G→T, rs3759387; *MVK*_S52NG→A, rs7957619; and 12inter_108521796T→G, rs9888325) were genotyped. SNPs were selected on the basis of 2 criteria: bioinformatics functional assessment (18) and LD structure. Assessment of the LD structure at the *KCTD10, MVK*, and *MMAB* loci with the use of data from the CEU (western European ancestry) population facilitated the selection of tag SNPs representing different LD blocks using an inclusion criterion of $r^2 > 0.8$. Intronic SNPs were also analyzed with MAPPER (22) to uncover potential allele-specific transcription factor binding sites and were manually checked for altered mRNA splice donor and acceptor sites and transversions affecting the poly-pyrimidine tract near splice acceptors. Genotyping was performed by using TaqMan assays with allele-specific probes on the ABIPrism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA) according to routine laboratory protocols (23). (*See* Supplemental Table 1 under "Supplemental data" in the online issue for a description of SNPs and ABI assay-on-demand ID identifiers.) The pairwise LD between SNPs was estimated as a correlation coefficient ($R$) in unrelated subjects by using the Helixtree software package (Golden Helix Inc, Bozeman, MT).

### Statistical analyses

SPSS software (version 16.0; SPSS Inc, Chicago, IL) was used for the statistical analyses. Differences in mean values were assessed by using analysis of variance with post hoc Bonferroni to

test for group differences. Categorical variables were compared by using Pearson's chi-square or the Fisher's exact tests. Potential confounding factors were age, sex, BMI, physical activity, smoking habit (current compared with never and past smokers), alcohol consumption (current compared with never and past drinkers), medication use (treatment of hypertension, diabetes, and hyperlipidemia and hormone treatment by women), prior CHD, and family relationships. Potential interactions between polymorphisms and dietary carbohydrates for HDL-cholesterol concentrations (as continuous variables) were tested by using the analysis of variance after further adjustment for total energy intake (in kcal). Corrections for multiple comparisons were made by using the Bonferroni technique so that *P* values were multiplied by the number of analyses performed. As a measure of the goodness-of-fit of the models, the square of the correlation coefficient among macronutrients was calculated. We further adjusted the models for intakes of saturated fat, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and protein (as continuous variables). Two-sided *P* values $<0.05$ were considered statistically significant. Data are presented as means ± SDs for continuous variables and as frequencies or percentages for categorical variables.

## RESULTS

Compared with women, men had higher systolic and diastolic BP measurements. As expected, HDL-cholesterol concentrations were lower in men than in women, whereas triglycerides were higher in men. Men had a higher prevalence of CHD and were more likely to receive treatment for hyperlipidemia than were women. No significant differences in other variables were observed (**Table 1**).

For all studied polymorphisms, there was no departure from the Hardy-Weinberg equilibrium ($P > 0.05$). (*See* Supplemental Table 2 under "Supplemental data" in the online issue for a presentation of the pairwise LD estimates expressed as correlation coefficients for all 8 SNPs.) Given that the SNP 12inter_108466061A→G was in strong LD (>0.9) with 12inter_108521796T→G, the latter SNP was excluded from further analyses. Because of the low genotype frequencies of homozygotes for the minor alleles, we analyzed 5 SNPs (*KCTD10*_V206VT→C, *KCTD10*_i5642G→C, *MMAB*_3U3527G→C, *MVK*_i851G→T, and *MVK*_S52NG→A) using 2 genotype categories (dominant model). The other 2 SNPs were analyzed by using 3 genotype categories (additive model). Considering the homogeneity between sex-specific genotype groups, men and women were pooled together for subsequent analyses.

For the SNPs *KCTD10*_i5642G→C and *MVK*_S52NG→A, homozygotes for the major allele (*G*) had lower HDL-cholesterol concentrations than did carriers of the minor alleles ($P = 0.005$ and $P = 0.019$, respectively) (**Table 2**). After Bonferroni correction, homozygotes for the i5642G allele still had lower HDL-cholesterol concentrations ($P = 0.015$), whereas differences between genotypes were marginally significant for the SNP *MVK*_S52NG→A ($P = 0.057$). For the SNP 12inter_108466061A→G, homozygotes for the minor allele (*G*) had higher total cholesterol and LDL-cholesterol concentrations than did *AG* subjects ($P = 0.024$ and $P = 0.029$, respectively), whereas no significant differences for these variables were found in homozygotes for the major allele (*A*) ($P > 0.2$ for both). For SNP *MMAB*_3U3527G→C, homozygotes for the major allele (*G*) had higher LDL-cholesterol concentrations than did those carriers of the minor allele ($P = 0.034$). No other significant associations were found between these studied SNPs and lipids. These differences between genotypes were no longer significant after Bonferroni correction for total cholesterol ($P = 0.090$) and LDL cholesterol ($P = 0.102$) (data not shown).

We next examined whether associations between SNPs and HDL-cholesterol concentrations were related to carbohydrate intake in this population. Because there were no significant

**TABLE 1**
Demographic and biochemical characteristics of men and women in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study ($n = 920$)

| | Men ($n = 441$) | Women ($n = 479$) | *P* |
|---|---|---|---|
| Age (y) | 49 ± 16[1] | 49 ± 16 | 0.814[2] |
| BMI (kg/m$^2$) | 28.6 ± 4.8 | 28.3 ± 6.4 | 0.314[2] |
| Systolic blood pressure (mm Hg) | 119 ± 14.9 | 113 ± 197.9 | <0.001[2] |
| Diastolic blood pressure (mm Hg) | 71 ± 8.9 | 66 ± 9.1 | <0.001[2] |
| Total cholesterol (mmol/L) | 4.95 ± 0.99 | 5.00 ± 1.07 | 0.435[2] |
| LDL cholesterol (mmol/L) | 3.22 ± 0.78 | 3.13 ± 0.86 | 0.069[2] |
| HDL cholesterol (mmol/L) | 1.07 ± 0.25 | 1.35 ± 0.36 | <0.001 |
| Triglyceride (mmol/L) | 1.68 ± 0.91 | 1.40 ± 0.81 | 0.001[2] |
| Current smokers [*n* (%)] | 37 (8) | 41 (9) | 0.918[3] |
| Current alcohol drinkers [*n* (%)] | 219 (50) | 229 (48) | 0.644[3] |
| Treatment of diabetes [*n* (%)] | 20 (5) | 23 (5) | 0.877[3] |
| Treatment of hypertension [*n* (%)] | 87 (20) | 80 (17) | 0.266[3] |
| Treatment to lower lipids [*n* (%)] | 24 (5) | 13 (3) | 0.043[3] |
| Hormone treatment [*n* (%)] | — | 96 (20) | <0.001[3] |
| Prior coronary heart disease [*n* (%)] | 43 (10) | 11 (2) | <0.001[3] |

[1] Mean ± SD (all such values).
[2] ANOVA.
[3] Chi-square test.

**TABLE 2**
Associations between single nucleotide polymorphisms and fasting lipid profiles in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study population ($n$ = 920)[1]

| | *TT* | *TC + CC* | *GG* | *GC + CC* | *AA* | *AG* | *TC* | *CC* | *GT + TT* | *AA + AG* | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *KCTD10*_V206VT→C | | | | | | | | | | | |
| Total cholesterol (mmol/L) | 4.96 ± 0.01 (634) | 5.01 ± 0.05 (286) | — | — | — | — | — | — | — | — | 0.488 |
| LDL cholesterol (mmol/L) | 3.16 ± 0.02 (634) | 3.21 ± 0.04 (286) | — | — | — | — | — | — | — | — | 0.348 |
| HDL cholesterol (mmol/L) | 1.22 ± 0.01 (634) | 1.22 ± 0.01 (286) | — | — | — | — | — | — | — | — | 0.954 |
| Triglycerides (mmol/L) | 1.57 ± 0.04 (634) | 1.45 ± 0.07 (286) | — | — | — | — | — | — | — | — | 0.185 |
| *KCTD10*_i5642G→C | | | | | | | | | | | |
| Total cholesterol (mmol/L) | — | — | 4.98 ± 0.03 (599) | 4.97 ± 0.05 (321) | — | — | — | — | — | — | 0.820 |
| LDL cholesterol (mmol/L) | — | — | 3.18 ± 0.03 (599) | 3.16 ± 0.04 (321) | — | — | — | — | — | — | 0.633 |
| HDL cholesterol (mmol/L) | — | — | 1.20 ± 0.01 (599) | 1.26 ± 0.01 (321) | — | — | — | — | — | — | 0.005 |
| Triglycerides (mmol/L) | — | — | 1.55 ± 0.04 (599) | 1.50 ± 0.06 (321) | — | — | — | — | — | — | 0.602 |
| 12inter_108466061A→G | | | | | | | | | | | |
| Total cholesterol (mmol/L) | — | — | 5.12 ± 0.06 (209)[2] | — | 4.97 ± 0.06 (217) | 4.92 ± 0.04 (494) | — | — | — | — | 0.030 |
| LDL cholesterol (mmol/L) | — | — | 3.29 ± 0.05 (209)[2] | — | 3.16 ± 0.05 (217) | 3.13 ± 0.03 (494) | — | — | — | — | 0.034 |
| HDL cholesterol (mmol/L) | — | — | 1.19 ± 0.02 (209) | — | 1.23 ± 0.01 (217) | 1.22 ± 0.01 (494) | — | — | — | — | 0.254 |
| Triglycerides (mmol/L) | — | — | 1.64 ± 0.08 (209) | — | 1.53 ± 0.08 (217) | 1.49 ± 0.05 (494) | — | — | — | — | 0.304 |
| *MMAB*_3U3527G→C | | | | | | | | | | | |
| Total cholesterol (mmol/L) | — | — | 5.03 ± 0.04 (482) | 4.92 ± 0.04 (438) | — | — | — | — | — | — | 0.075 |
| LDL cholesterol (mmol/L) | — | — | 3.22 ± 0.03 (482) | 3.12 ± 0.03 (438) | — | — | — | — | — | — | 0.034 |
| HDL cholesterol (mmol/L) | — | — | 1.21 ± 0.01 (482) | 1.23 ± 0.01 (438) | — | — | — | — | — | — | 0.342 |
| Triglycerides (mmol/L) | — | — | 1.59 ± 0.05 (482) | 1.47 ± 0.05 (438) | — | — | — | — | — | — | 0.161 |
| *MVK*_m751T→C | | | | | | | | | | | |
| Total cholesterol (mmol/L) | 5.02 ± 0.05 (312) | — | — | — | — | — | 4.93 ± 0.04 (441) | 5.02 ± 0.07 (167) | — | — | 0.406 |
| LDL cholesterol (mmol/L) | 3.19 ± 0.04 (312) | — | — | — | — | — | 3.14 ± 0.03 (441) | 3.22 ± 0.05 (167) | — | — | 0.468 |
| HDL cholesterol (mmol/L) | 1.21 ± 0.01 (312) | — | — | — | — | — | 1.23 ± 0.01 (441) | 1.21 ± 0.02 (167) | — | — | 0.556 |
| Triglycerides, mmol/L | 1.60 ± 0.06 (312) | — | — | — | — | — | 1.49 ± 0.05 (441) | 1.52 ± 0.09 (167) | — | — | 0.476 |

*(Continued)*

**TABLE 2** (*Continued*)

| | *TT* | *TC + CC* | *GG* | *GC + CC* | *AA* | *AG* | *TC* | *CC* | *GT + TT* | *AA + AG* | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *MVK*_i851G→T | | | | | | | | | | | |
| Total cholesterol (mmol/L) | — | — | 5.00 ± 0.04 (513) | — | — | — | — | — | 4.94 ± 0.04 (407) | — | 0.327 |
| LDL cholesterol (mmol/L) | — | — | 3.20 ± 0.03 (513) | — | — | — | — | — | 3.14 ± 0.03 (407) | — | 0.282 |
| HDL cholesterol (mmol/L) | — | — | 1.21 ± 0.01 (513) | — | — | — | — | — | 1.23 ± 0.01 (407) | — | 0.452 |
| Triglycerides (mmol/L) | — | — | 1.52 ± 0.05 (513) | — | — | — | — | — | 1.55 ± 0.06 (407) | — | 0.672 |
| *MVK*_S52NG→A | | | | | | | | | | | |
| Total cholesterol (mmol/L) | — | — | 4.98 ± 0.03 (725) | — | — | — | — | — | — | 4.97 ± 0.06 (195) | 0.898 |
| LDL cholesterol (mmol/L) | — | — | 3.19 ± 0.02 (725) | — | — | — | — | — | — | 3.12 ± 0.05 (195) | 0.287 |
| HDL cholesterol (mmol/L) | — | — | 1.21 ± 0.01 (725) | — | — | — | — | — | — | 1.26 ± 0.02 (195) | 0.019 |
| Triglycerides (mmol/L) | — | — | 1.54 ± 0.04 (725) | — | — | — | — | — | — | 1.52 ± 0.08 (195) | 0.894 |

[1] All values are means ± SEs; *n* in parentheses. Differences in mean values were assessed by ANOVA with post hoc Bonferroni multiple comparisons. Multivariate *P* values were adjusted for age, sex, BMI, physical activity, smoking habit, alcohol consumption, medication use, prior coronary heart disease, and family relationships.

[2] Significantly different from *AG*, $P < 0.05$ (Bonferroni post hoc test).

differences in dietary intake according to genotype groups (data not shown), we investigated whether interactions between carbohydrate intake and genes (*KCTD10, MVK*, and *MMAB*) could modulate the observed associations with HDL-cholesterol concentrations. We dichotomized carbohydrate intake according to the median value (<231 compared with ≥231 g/d). A significant gene-diet interaction was found ($P = 0.007$); carriers of the *C* allele at SNP *KCTD10*_V206VT→C who consumed diets containing high amounts of carbohydrate had lower HDL-cholesterol concentrations than did subjects homozygous for the *T* allele ($P = 0.011$), whereas a trend toward higher concentrations was observed in carriers of the *C* allele who consumed diets with low amounts of carbohydrate ($P = 0.056$) (**Figure 1**). A significant gene-diet interaction was also observed for the SNP *KCTD10*_i5642G→C ($P = 0.038$); *GG* subjects who consumed diets with high amounts of carbohydrate had lower HDL-cholesterol concentrations than did carriers of the *C* allele ($P < 0.001$). However, no significant differences based on genotype were found among subjects who consumed diets with low amounts of carbohydrate ($P > 0.5$) (**Figure 2**). Finally, a significant gene-diet interaction was observed for the SNP *MMAB*_3U3527G→C ($P < 0.001$); *GG* subjects consuming diets with low amounts of carbohydrate had higher HDL-cholesterol concentrations than did carriers of the *C* allele ($P = 0.001$), whereas lower concentrations were seen in *GG* subjects who consumed diets with high amounts of carbohydrate ($P = 0.009$) (**Figure 3**). Because the carbohydrate intake was highly correlated with intakes from saturated fat, MUFAs, PUFAs, and protein ($r = 0.752-0.841$, $P < 0.001$), models were additionally adjusted for these nutrients. Further adjustment for these nutrients had little effect on gene-diet interactions for the SNPs *MMAB*_3U3527G→C and KCTD10_V206VT→C ($P < 0.001$ and $P = 0.001$, respectively), whereas these interactions were slightly stronger for the SNP *KCTD10*_i5642G→C ($P = 0.033$). A marginally significant gene-diet interaction was found for the SNP *MVK*_S52NG→A ($P = 0.055$); subjects homozygous for the S52NG allele had lower HDL-cholesterol concentrations only if they consumed diets with high amounts of carbohydrate ($P < 0.001$) (**Table 3**).

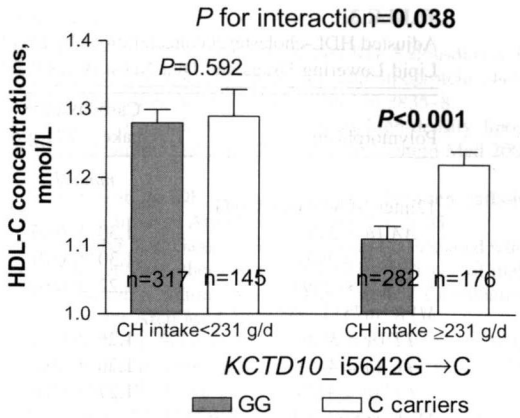


**FIGURE 2.** Mean (±SE) effect of *KCTD10*_i5642G→C genotype on HDL-cholesterol (HDL-C) concentrations by carbohydrate (CH) intake in Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study participants ($n = 920$). *P* values were adjusted by ANOVA.

## DISCUSSION

This study provides the first evidence that genetic variation at *KCTD10, MVK*, and *MMAB* genes modulates plasma HDL-cholesterol concentrations depending on dietary carbohydrates. Results indicate that homozygotes for major alleles at SNPs *KCTD10*_i5642G→C, *MMAB*_3U3527G→C and *MVK* (i851G→T and S52NG→A) displayed lower HDL-cholesterol concentrations than carriers of the minor alleles, only if they consumed diets rich in carbohydrates. A significant gene-diet interaction was observed for SNP *KCTD10*_V206VT→C; carriers of the minor allele showed lower HDL-cholesterol concentrations than did homozygotes for the major allele, only in subjects who consumed diets high in carbohydrates. Therefore, homozygotes for major alleles at these SNPs and carriers of the 206V allele showed an interaction with dietary carbohydrates, which lowers plasma HDL-cholesterol concentrations, whereas carriers of the minor alleles and homozygotes for the V206 allele were resistant to carbohydrate-induced decreases in HDL-cholesterol concentrations. Overall, these findings suggest that dietary habits may modulate the contributions of *KCTD10,*

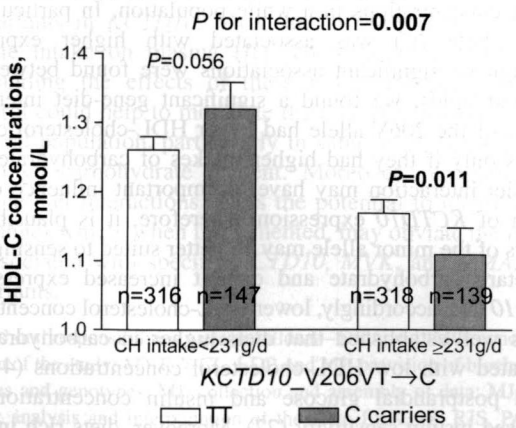


**FIGURE 1.** Mean (±SE) effect of *KCTD10*_V206VT→C genotype on HDL-cholesterol (HDL-C) concentrations by carbohydrate (CH) intake in Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study participants ($n = 920$). *P* values were adjusted by ANOVA.

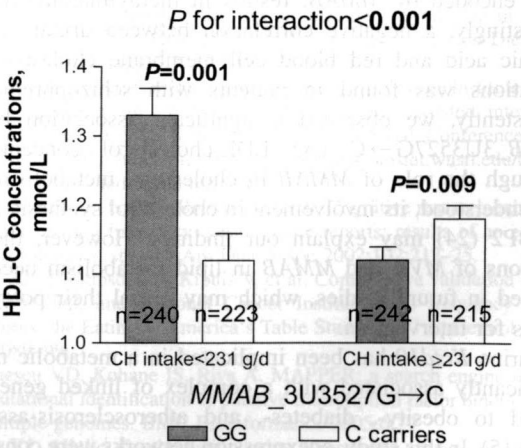


**FIGURE 3.** Mean (±SE) effect of *MMAB*_3U3527G→C genotype on HDL-cholesterol (HDL-C) concentrations by carbohydrate (CH) intake in Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study participants ($n = 920$). *P* values were adjusted by ANOVA.

**TABLE 3**
Adjusted HDL-cholesterol concentrations by *KCTD10, MVK*, and *MMAB* loci and carbohydrate intake in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study population ($n$ = 920)[1]

| Polymorphism | Carbohydrate intake <231 g/d | $P$ | Carbohydrate intake ≥231 g/d | $P$ | $P$ for interaction |
|---|---|---|---|---|---|
| | *mmol/L* | | *mmol/L* | | |
| 12inter_108466061A→G | | | | | |
| *AA* ($n$ = 217) | 1.30 ± 0.03 | 0.051 | 1.17 ± 0.02 | 0.569 | 0.104 |
| *AG* ($n$ = 494) | 1.30 ± 0.01 | | 1.14 ± 0.01 | | |
| *GG* ($n$ = 209) | 1.22 ± 0.03 | | 1.16 ± 0.02 | | |
| *MVK*_m751T→C | | | | | |
| *TT* ($n$ = 312) | 1.26 ± 0.02 | 0.342 | 1.15 ± 0.02 | 0.925 | 0.690 |
| *TC* ($n$ = 441) | 1.30 ± 0.02 | | 1.16 ± 0.01 | | |
| *CC* ($n$ = 167) | 1.27 ± 0.03 | | 1.16 ± 0.02 | | |
| *MVK*_i851G→T | | | | | |
| *GG* ($n$ = 513) | 1.29 ± 0.01 | 0.640 | 1.13 ± 0.01 | 0.034 | 0.124 |
| *T* carriers ($n$ = 407) | 1.27 ± 0.02 | | 1.18 ± 0.01 | | |
| *MVK*_S52NG→A | | | | | |
| *GG* ($n$ = 725) | 1.28 ± 0.03 | 0.811 | 1.13 ± 0.01 | <0.001 | 0.055 |
| *A* carriers ($n$ = 195) | 1.29 ± 0.03 | | 1.24 ± 0.02 | | |

[1] All values are means ± SEs. Differences in mean values were assessed by ANOVA. Multivariate $P$ values were adjusted for age, sex, BMI, physical activity, smoking habit, alcohol consumption, medication use, prior coronary heart disease, family relationships, and total energy intake.

*MVK*, and *MMAB* polymorphisms to the genetic susceptibility toward lowering HDL-cholesterol concentrations.

Significant associations were found between the SNPs 12inter_108466061A→G and *MMAB*_3U3527G→C and LDL-cholesterol concentrations. However, these associations were stronger for HDL-cholesterol concentrations; homozygotes for the major alleles at the SNPs *KCTD10*_i5642G→C and *MVK*_252NG→A had lower concentrations.

The *MVK* gene, which encodes the catalyst of an early step in cholesterol biosynthesis, has been implicated to affect HDL-cholesterol concentrations in 2 recent GWAS (7, 8), which supports our findings. However, others found no significant associations between the *MVK*_S52NG→A SNP and HDL-cholesterol concentrations (16). These discrepancies may be due to differences in age, HDL-cholesterol concentrations, and lifestyle (particularly smoking habit) across populations. In contrast, deficiency of cob(I)alamin adenosyltranferase, an enzyme encoded by *MMAB*, results in methylmalonic aciduria. Interestingly, a negative correlation between urinary methylmalonic acid and red blood cell membrane cholesterol concentrations was found in patients with schizophrenia (14). Consistently, we observed a significant association between *MMAB*_3U3527G→C and LDL-cholesterol concentrations. Although the role of *MMAB* in cholesterol metabolism is not well understood, its involvement in cholesterol synthesis through SREBP2 (24) may explain our findings. However, the exact functions of *MVK* and *MMAB* in lipid metabolism need to be clarified in future studies, which may reveal their potential as targets for intervention.

Murine *Kctd10* has been implicated in a metabolic network significantly associated with a complex of linked genetic loci related to obesity-, diabetes-, and atherosclerosis-associated traits (15). In this study, coexpression networks were constructed from gene activity data derived from liver and adipose tissue collected from a segregating mouse population and identified several subnetworks associated with metabolic traits. Particularly, *Kctd10* was included in a network enriched for expression traits supported as causal for ≥1 of the following 6 metabolic traits: abdominal fat mass, weight, plasma insulin concentrations, free fatty acids, total plasma cholesterol concentrations, and aortic lesion size. Therefore, by extension, the involvement of human *KCTD10* in this metabolic network coupled with our results supports its association with HDL-cholesterol concentrations.

Importantly, this is the first study to show an interaction between novel variants at the *KCTD10* and *MMAB* genes with carbohydrate intake. The mechanism by which these polymorphisms may contribute to the observed interactions is unknown. Given that 3 of the analyzed SNPs map to noncoding regions and are members of large LD blocks, the likelihood that these SNPs represent a functional mutation is low. However, the presence of transcriptional enhancers and other regulatory elements, observed frequently in intronic regions (25), could explain our findings. Interestingly, Dixon et al (26) reported that the *KCTD10*_V206VT→C SNP was associated with *KCTD10* mRNA concentrations in a white population. In particular, the minor allele (*C*) was associated with higher expression. Although no significant associations were found between this SNP and lipids, we found a significant gene-diet interaction; carriers of the 206V allele had lower HDL-cholesterol concentrations only if they had higher intakes of carbohydrates. This gene-diet interaction may have an important influence on regulation of *KCTD10* expression. Therefore, it is plausible that carriers of the minor allele may be better suited to sensing levels of dietary carbohydrate and exhibit increased expression of *KCTD10* and, accordingly, lower HDL-cholesterol concentrations.

It is well-established that diets higher in carbohydrates are associated with low HDL-cholesterol concentrations (4–6), elevated postprandial glucose and insulin concentrations, and decreased insulin sensitivity (27). Moreover, diets rich in carbohydrates are associated with an increased risk of atherosclerosis through the insulin-mediated activation of the renin-angiotensin system, growth factors, and cytokines (28). Overall, our data

support a synergistic relation between genes and diet by which tailored dietary recommendations targeted at increasing HDL-cholesterol concentrations may modulate the genetic expression of several *KCTD10, MVK*, and *MMAB* genetic variants. The potential mechanisms that underlie these gene-diet interactions may include dietary influences on HDL production and transport rates and catabolic processes. Of note, compared with dietary interventions, similar increases in HDL-cholesterol concentrations have been reported in patients with low HDL-cholesterol concentrations after drug therapy (5–20%). Therefore, the present study provides proof-of-concept for the potential application of genetics in the context of personalized nutritional recommendations for cardiovascular disease prevention (29).

Despite the evidence, our data should be interpreted with caution. The cross-sectional design of this study may weaken or distort any true relation between *KCTD10, MVK*, and *MMAB* and carbohydrate intakes. In this regard, large prospective studies with a long period of follow-up are required to clarify the directionality of these associations. Because carbohydrate intake is only part of a larger landscape of dietary intake, we further adjusted for intake of other macronutrients. Although these adjustments had little effect on the results, this adjustment was likely incomplete because of residual confounding by imprecisely measured dietary factors. The exploratory nature of the present study and the large number of comparisons necessitates further studies in other larger populations and ethnic groups, particularly those with low HDL-cholesterol concentrations. Consideration of the variability of minor allele frequencies of the examined SNPs compared with those reported from other populations with different ethnic backgrounds likely will reveal variants exerting a greater effect on HDL-cholesterol concentrations and/or a greater susceptibility to interactions with environmental factors. According to HapMap data, minor allele frequencies (MAFs) in our study population are higher for the 7 SNPs with data than for those reported in Chinese and Japanese populations and similar to those observed in Europeans. The Yoruba population from Africa presents MAFs similar to or greater than MAFs in our population. For example, the minor A allele of rs3759387 shows MAFs of 24% in European, 44% in African, and 15–17% in Asian populations, respectively.

In conclusion, the present study showed an interaction between novel variants in *KCTD10, MVK*, and *MMAB* genes and carbohydrate intake on plasma HDL-cholesterol concentrations. Understanding the effects of these polymorphisms on HDL cholesterol could help to modulate the risk of atherosclerosis in the general population, particularly in subjects consuming diets with a high carbohydrate content. Moreover, recognition of these gene-diet interactions offers the potential to identify lifestyle changes which, when implemented, may obviate the risk of CHD associated with specific *KCTD10, MVK*, and *MMAB* genetic variants.

The authors' responsibilities were as follows—DKA and JMO: conception and design of the study; MYT, Y-CL, LDP, and MJ: provision of biochemical phenotypes and genotypes; MJ: collection and assembly of data; MJ, LDP, and JMO: analysis and interpretation of the data; EKK, IB, RJS, PA, and MP: statistical expertise; JMO, LDP, and MJ: writing of the manuscript draft; RJS, DKA, EKK, PA, C-QL, LDP, CES, and Y-CL: critical review of the manuscript; and DKA, JMO, and MJ: funding. None of the authors had a personal or financial conflict of interest.

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